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Use of quantitative hepatitis B surface antigen levels in the follow-up of HBV-Infected patients with Genotype D

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Abstract

To determine the role of quantitative HBsAg levels in follow up of chronic HBV treatment and investigate the relationship between qHBsAg, HBV DNA levels and liver histopathology. 74 CHB patients with genotype D visited our Infectious Diseases outpatient clinic were included into the study. Patients were grouped according to treatment status; Group-I (patients new to treatment, 31 patients) and group-II (patients with long-term treatment history). All group-I patients had their serum HBsAg titers (qHBsAg) measured before treatment, at 3 and 6-month time periods after treatment. Group-II consisted of 43 CHB patients who had been on anti-viral treatment for at least 5 years. qHBsAg levels were measured and compared to that of patient in group II. Patients from group I were divided into two groups according to HBeAg positivity. The mean qHBsAg and HBVDNA levels and fibrosis scores were statistically higher (p=0.002, p =0.034, p=0.002) in HBeAg positive patients. For all patients in group-I, a positive correlation was found between qHBsAg and HBV DNA levels before treatment (p=0.003). Serum qHBsAg and HBVDNA levels that were measured before treatment, 3-month and 6-month after treatment were statistically different (p< 0.05) from each other. Group-II patients were classified according to YMDD mutations and virologic breakthrough during treatment. Serum qHBsAg levels of patients with YMDD mutations and virologic breakthrough were found to be statistically higher (p=0.010) than those who did not although these patients were receiving potent antivirals. Quantitative HBsAg levels may differ among chronic HBV patients according to their treatment protocols and duration. In patients who are new to treatment, HBeAg positive patients may have statistically higher levels of qHBsAg. Moreover, this difference was also observed when patient who are new to treatment were compared to those who had been under treatment for a longer time. For clinicians, qHBsAg levels should not be employed only in patients undergoing interferon therapy but also those undergoing antiviral therapy as well..

Keywords: Chronic hepatitis B, hepatitis B surface antigen, genotype

Introduction

In order to diagnose HBV infection, practitioner has resorted to HBsAg for years. On the one hand this antigen conserves its diagnostic benefit; on the other hand it has acquired a particular recognition due to progress in quantitative approaches [1]. As a result, HBsAg became more relevant along time [1]. Despite the fact that HBV DNA negativity is considered as the consequent target for patients, HBsAg seroconversion remains the main conclusion for patients suffering from CHB [1-3]. Due to the improvement of qHBsAg and cccDNA metrics, potential way rose to the surface in order to develop new treatment practices. Moreover, due to these findings that permitted the actual developed practices, a substantial increase in seroclearance of HBsAg is forecasted soon.

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It has been revealed that the relevance of co-operative use of qHBsAg and HBV-DNA as a diagnosis technique and monitoring of CHB treatment. A cooperative use of qHBsAg<1000 IU/mL and HBV-DNA <2000 IU/mL can recognize inactive carriers [1,4]. qHBsAg levels vary during the natural history of Chronic Hepatitis B infection and progressively decline from the immune tolerance to the inactive phase [1,4,5]. The application of qHBsAg in CHB patients may influence relevant interdependent information to HBV DNA during treatment and help to forecast the treatment issue.The aims of this study are a) to determine the role of qHBsAg levels in follow up of chronic HBV treatment and b) to investigate the relationship between qHBsAg, HBV DNA levels and liver histopathology.

Material and Methods

A total number of 74 patients diagnosed with chronic hepatitis B by clinical, biochemical, serological and histopathological methods who referred to our outpatient Clinic for Infectious Diseases and

Clinical Microbiology located in GATA Haydarpasa Training Hospital between June 1, 2014 and December 31, 2014 were enrolled into our study. Based on the biopsy results obtained from reports in accordance to the Modified Ishak Scoring System, scores of HAI 9 and over was accepted as patients with a severe level of inflammation. During histopathological studies were carried out, patients with a fibrosis stage III and over were considered as cases with an advanced degree of fibrosis. Serum qHBsAg levels were measured by chemiluminescent microparticle immunoassay (CMIA) among patients who were included in the study, using an ARCHITEC (Abbott Laboratories) device, and on the other hand a quantitative PCR method (Applied Biosystems, ABI 7500 Real Time PCR System) was utilized to determine HBV-DNA levels. HBV genotyping was performed in all patients (Bosphore HBV Genotype Detection Kit, Anatolia Geneworks, Istanbul, Turkey). before antiviral therapy. In patients who are under long term therapy, genotyping was performed in stored sera collected before beginning of therapy. Regarding the 2014-57 numbered research project, this study was evaluated by the 25th Ethic Council, dated May 15, 2014 by the Turkish Republic Presidency of the General Staff GATA HEH and was decided appropriate in compliance to ethical principles due to the scope and purpose, method and approach and therefore the study was launched.

Inclusion Criteria of the Study

A total number of 74 chronic hepatitis B patients with genotype D referred to GATA Haydarpasa Training Hospital, Outpatient Clinic of Infectious Diseases and Clinical Microbiology who were diagnosed with CHB by clinical, biochemical, serological and histopathological methods and receiving Peginterferon alfa-2a or oral antiviral therapies, were included into the study after the obtention of the permission from the patient via a written patient consent form. Patients were divided into two groups such as First-Time Treated Patients Group and Long-Term Treated Patients Group. Patients in the first group consisted from 31 naïve CHB patients who never received a therapy before. We planned to assess the antiviral efficacy together with HBsAg titers studied in patient serums collected during the 3rd and 6th months of the ongoing therapy, prior the therapy in this patient group who received oral antiviral and interferon treatments. For this purpose, cases who did not receive an antiviral therapy for the last 2 years and who were above 18 years old and was found HBsAg positive for at least 6 months were included in this group of patients. There were 43 patients with CHB in the other group of patients included in our study whereas these patients received an antiviral therapy for at least five years. Serum samples collected from this group of patients were also included in the study, their qHBsAg levels were investigated after a long-term therapy, and the results were compared with the results obtained from patients who we just began to treat. The information of patients enrolled in the study was derived from the hospital computer operating system and outpatient follow-up archives.

Exclusion Criteria of the Study

As an exclusion criteria in a group of patients who newly began to receive therapy (First-Time Treated Patients Group), patients under 18 years of age, and who have another viral etiology apart from HBV for liver disease, and individuals with a HCV, HIV and HDV co-infection, patients who previously received an antiviral therapy and patients with a severe systemic condition, hemochromatosis, Wilson's Disease, toxic and alcoholic hepatitis, malignity, pregnancy, liver transplantation, anemia or hematological disease were excluded from the study. As an exclusion criteria in a group of patients who were receiving a long-term therapy (Long-Term Treated Patients Group), patients under 18 years of age, and who have another viral etiology apart from HBV and individuals with a HCV, HIV and HDV co-infection, patients who previously received an antiviral therapy for a period less than 5 years, and patients with a severe systemic condition, hemochromatosis, Wilson's Disease, toxic and alcoholic hepatitis, cirrhosis, malignity, pregnancy, liver transplantation, anemia or hematological disease were excluded from the study. Furthermore, patients with missing data regarding descriptive, demographic and clinic course were also excluded from the study.

Distribution of the patient groups in the study are displayed in Figure 1.

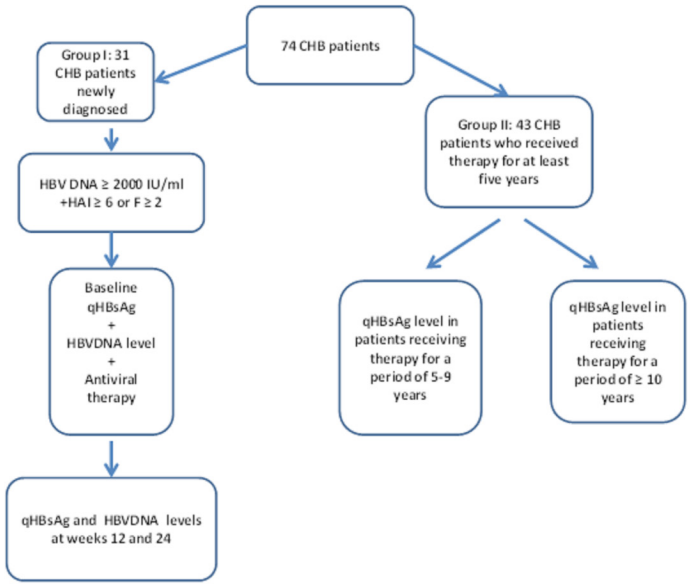


Figure 1. Distribution of the patient groups in the study are displayed in

Biochemical and Virology Laboratory Measurements

Complete blood count, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gammaglutamyl transferase (GGT), total Bilirubin, albumin (Alb), HBV DNA, HBeAg, Anti-HBe were studied as routine procedures in the biochemistry and microbiology laboratories by using standard methods. An Architect HbsAg quantitative assay (Abbott Diagnostics, Sligo Ireland) kit was used for HBs-Ag titer measurement (HBsAg quantitation range 0.05-250 IU/ml). Patient serum samples with values that exceed 250 IU/ml were reported after re-calculated as an "IU/ml" unit when diluted at a ratio of 1:500 due to automatic dilution protocol.

Statistical analysis

A SPPS 20.0 package program (SPSS Inc., Chicago, Illinois, USA) was used to assess patient data. Percentage, average, standard deviation, media, minimum and maximum values were used as descriptive statistics. A Chi-Square Test was applied to statistically compare intermittent variables. However, a Kolmogorov Smirnov test was used to investigate the compatibility of continuous variables to normal distribution. If results were compatible to a normal distribution, Student t test, and if not, Mann Whitney U test was performed. Friedman test was applied to demonstrate whether

a connection was present or not in repetitive measurements. Bonferroni corrected variance analysis was applied during the comparison of more than two groups, if they were compatible with a normal distribution. Bonferroni corrected Wilcoxon test was used in double comparisons to determine groups that created a discrepancy. Confidence interval was found 95% while statistical significance was $p<0.05$.

Results

Patients' Characteristics

A total number of 74 patients who were receiving an oral antiviral or pegylated interferon alfa-2a treatment due to a CHB infection were enrolled in the study. Average age of patients was 44.04 ± 15.652. A number of 28 (37.84%) female and 46 (62.16%) male patients were included in the study. In genotype analysis, genotype D was detected in all patients. First of all, patients were divided into two groups such as patients who newly started therapy and patients who were receiving therapy for a long period. A statistically significant difference was determined between these groups of patients due to qHBsAg levels ($p=0.001$) while no significant difference was determined due to baseline fibrosis stage and HAI score ($p>0.05$). Demographic, histopathological and laboratory characteristics of patients included in our study are displayed in Table 1. Mean qHBsAg level in patients who newly started therapy (14373 ± 18435.51 IU/ml), was found higher regarding a statistical significant ratio when compared with patients who had been on treatment between 5-9 years (4422.33 ± 6053.56 IU/ml) or patients who had received treatment for more than 10 years (1191.49 ± 2336.88 IU/ml) ($p=0.001$).

Table 1. Clinical and laboratory characteristics of naive patients and CHB patients under long term therapy

Patient characteristics	Naive patients with CHB (n=31)	CHB patients receiving therapy since 5 years or more (n=43)	p
Age	36,81±13,36	49,26±15,227	**0,001**
Gender, male (total)	18 (31)	28 (43)	0,537
qHBsAg (IU/ml)	14373±18435,51	3065,581±4493,082	**0,001**
qHBsAg(log10 IU/ml)	3,87	2,79	**0,001**
HAI score	7,48±2,63	6,80±3,13	0,322
HAI skoru <9	19	30	**0,215**
HAI skoru ≥9	12	10	
Fibrosis stage	2,42±1,205	2,50±1,35	0,625
Fibrosis <3	15	15	**0,234**
Fibrosis ≥3	16	25	
Genotype D	31/31	43/43	

Clinical and laboratory features of patients with chronic hepatitis B who were newly diagnosed and started to receive therapy

Eighteen (58.1%) of the patients who were newly diagnosed and started to receive therapy were males and thirteen (41.9%) were females. The average age of patients was 36.81 ± 13.36. The average age of male patients was determined 31.72 ± 12.73 while this value was 43.85 ± 11.13 for females. Eight of the patients mentioned above were receiving a lower genetic barrier drug (19.4%) patients were receiving lamivudine and 2 (6.5%) patients were receiving telbivudine] while twenty one patients were receiving a higher genetic barrier oral antiviral drug [8 (25.8%) patients were receiving tenofovir, 13 (41.9%) patients were receiving entecavir] and two (6.5%) patients were receiving a pegylated interferon alpha-2a therapy. Interferon therapy was also applied to one of the patients who was currently receiving entecavir therapy. Pre-therapeutical mean HBV DNA and qHBsAg levels of patients were determined respectively, 655.320.263 IU/ml (8,82 log10 IU/ml) and 14373,96 IU/ml (4,16 log10 IU/ml). The qHBsAg levels of 3 (9.7%) patients was found below 3 log IU/ml (1000 IU/ml) while qHBsAg levels of 13 (41.9%) patients was found between 3-4 log IU/ml (1000-10000 IU/ml) and over 4 log IU/ml in 15 (48.4%) patients. Similar mean qHBsAg levels were determined in females and males when the all qHBsAg levels of patients were considered in a logarithmic manner (Female mean qHBsAg: 3,83 log 10 IU/ml; Male mean qHBsAg: 3,88 log 10 IU/ml; p=0,826).

While pre-treatment ALT, AST median values were found, respectively 62U/L (15-441); 43U/L (15-254) in CHB patients who newly started to receive therapy; mean albumin, total bilirubin, platelet and PT levels were respectively 4.22 ± 0.37g/dl; 0.76 ± 0.34mg/dl; 222 x 10^3 ± 65 x 10^3/µL; 13.71 ± 1.17 s. The relationship between pre-treatment baseline qHBsAg level and total bilirubin was investigated by Pearson correlation analysis and no statistically significant correlation was determined ($p>0.05$). Once again, no statistically significant correlation was determined between HBV DNA and ALT, AST values ($p>0.05$).

Correlation of qHBsAg and HBV DNA in patients with chronic hepatitis B who were newly started to receive therapy

At the beginning of the therapy, a statistically significant positive correlation was determined between qHBsAg and HBV DNA levels ($p=0.003$, $r=0,616$). At the 3rd and 6th months of therapy, a statistically significant correlation was determined between qHBsAg and HBV DNA levels ($p=0.0001$, $r=0,0,861$; $p=0.003$, $r=0,692$).

After patients were studied in two groups according to their HBV DNA levels, as cases with a value over and below 2.000.000 IU/ml, even though we determined a high qHBsAg level in the group of patients with a higher HBV DNA level, no statistically significant difference was determined between both two groups, regarding their qHBsAg levels ($p=0.94$). We also found statistically significant and higher ALT, HBsAg/HBV DNA (x log 10 IU/ml) and HAI levels in the group of patients with a high HBV DNA level ($p<0.05$). Groups of patients who newly started to receive a therapy according to their HBV DNA level are displayed in Table 2.

Table 2. Comparison of the naive CHB patient groups according to HBV DNA levels

	Group 1 (n=18) (HBV DNA;0-1999999 IU/ml)	Grup 2 (n=13) (HBV DNA; ≥2000000 IU/ml)	P value
ALT (U/ml)	49,94±27,61	168,92±119,60	0,001
qHBsAg(IU/ml)	14167,94±18420,15	14659,22±19205,21	0,944
qHBsAg(xlog 10 IU/ml)	3,859 ± 0,587	3,873±0,574	0,853
qHBsAg/HBVDNA(xlog10 IU/ml)	0,845 ± 0,175	0,505±0,068	0,001
HAI	6,61 ± 2,75	8,69 ± 1,97	0,020
Fibrosis	2,33 ± 1,085	2,54 ± 1,39	0,665

Comparison of the qHBsAg, HBV DNA, ALT and other laboratory results of naive cases according to liver fibrosis levels

Once we studied the patients in the both two groups who were divided as patients at a stage below III (0-II) and stage III and over (III-VI) regarding their clinic and laboratory findings, and in accordance to the fibrosis stage of CHB patients who newly started to receive a therapy; we found no statistically significant difference between baseline qHBsAg and ALT levels between both two groups. In the meantime, a statistically significant difference was determined in patients regarding HBV DNA, platelet, albumin and RDW levels between both two groups (p=0.001; p=0.035; p=0.010; p=0.021). Pre-treatment clinic and laboratory data of CHB patients who newly began to receive therapy in terms of histopathological stage are compared in Table 3 and Table 4.

Table 3. Comparison of the naive cases with liver fibrosis scores 0-2 and 3-6

	Fibrosis	N	Mean value	SD	P
Age (year)	Fibrosis<3	15	36,87	13,29	0,652
	Fibrosis ≥3	16	36,75	13,86	
Gender (male)	Fibrosis <3	8(15)	-	-	-
	Fibrosis ≥3	10(16)	-	-	
WBC	Fibrosis <3	15	7,26	1,83	0,165
	Fibrosis ≥3	16	6,32	1,84	
Hemoglobin(g/dl)	Fibrosis <3	15	14,08	1,64	0,077
	Fibrosis≥3	16	15,23	1,81	
Platelet(/mm3)	Fibrosis <3	15	248,60	75,970	0,035
	Fibrosis ≥3	16	198,25	43,69	
MPV (fL)	Fibrosis <3	15	7,704	1,18	0,057
	Fibrosis ≥3	16	7,706	0,70	
RDW	Fibrosis <3	15	13,067	1,25	0,021
	Fibrosis ≥3	16	11,96	1,24	
Blood glucose (mg/dl)	Fibrosis <3	15	85,27	10,22	0,061
	Fibrosis ≥3	16	96,19	21,29	
Total bilirubin	Fibrosis<3	15	0,74	0,337	0,535
	Fibrosis≥3	16	0,77	0,365	
Albumin (gr/dl)	Fibrosis<3	15	4,36	0,227	0,010
	Fibrosis≥3	16	4,09	0,442	
ALT(U/L)	Fibrosis<3	15	87	88,31	0,489
	Fibrosis≥3	16	111,88	108,78	
AST(U/L)	Fibrosis<3	15	58,80	52,58	0,751
	Fibrosis≥3	16	65,13	57,46	
Urea	Fibrosis<3	15	26,93	5,48	0,133
	Fibrosis≥3	16	31,06	8,99	
PT	Fibrosis<3	15	13,49	0,97	0,305
	Fibrosis≥3	16	13,92	1,32	
INR	Fibrosis<3	15	1,013	0,067	0,965
	Fibrosis≥3	16	1,015	0,068	

WBC, white blood cell; ALT, alanine aminotransferase; AST, aspartate aminotransferase; MPV, mean platelet volume; RDW, red blood cell distribution width; PT, prothrombin time

Table 4. Comparison of the naive cases with liver fibrosis scores 0-2 and 3-6

	Fibrosis	N	Mean /Median*	SD/ (min-max)*	P
qHBsAg(IU/ml)*	Fibrosis<3	15	10263	(293-73053)*	0,574
	Fibrosis≥3	16	9238	(1789-80057)*	
HBV DNA*	Fibrosis<3	15	797246*	(2015-10470000000)*	**0,001**
	Fibrosis≥3	16	2394000*	(2466-142800000)*	
HBV DNA (x log 10 IU/ml)	Fibrosis<3	15	5,94	2,10	0,454
	Fibrosis≥3	16	6	1,64	
HBsAg/HBV DNA(log)	Fibrosis<3	15	0,69	0,20	0,931
	Fibrosis≥3	16	0,70	0,24	

*** qHBsAg ve HBV DNA levels were not compatible with normal distribution for this reason, Mann-Whitney U Test is utilized for these parameters and * refers "median", "min-max" values of these parameters**

Comparison of demographic and laboratory results of CHB patients according to the status of HBeAg positivity

Patients in the same group who newly started to receive therapy were divided into two groups for assessment, regarding their HBeAg status, as positive or negative. The average age of HBeAg negative patients was found higher than the average age of HBeAg positive patients (p=0.01). No significant differences were determined between both groups of patients regarding white blood cells, hemoglobin, platelet, ALT, AST, total bilirubin, albumin, urea, creatinine, PT and INR. However, HBV DNA and qHBsAg levels and the stage of fibrosis was determined statistically significantly higher in the HBeAg positive group (p<0.005). Demographic, histopathologic and laboratory characteristics of HBeAg positive and negative patients are displayed in Table 5.

Table 5. Comparison of CHB patients according to the status of HBeAg positivity or negativity

Patient characteristics	Naive CHB patients		P value
	HBeAg negative (25)	**HBeAg positive (6)**	**P value**
Age	39,32±13,244	26,33±8,11	0,01
Gender, Male (%)	13 (%52)	5 (%83,3)	_
WBC(x10³/µL)	6,91±1,984	6,188±1,266	0,285
Hemoglobin (g/dl)	14,424±1,78	15,717±1,63	0,124
Platelet(x10³/µL)	222,36±71,98	223,67±30,696	0,102
ALT (U/L)*	61 (15-441)*	109,50 (49-276)*	0,117
AST(U/L)	62,24±59,63	61,33±25,21	0,322
Tot. Bilirübin (mg/dl)	0,702±0,30	1,002±0,44	0,080
Albümin (g/dl)	4,34±0,277	4,50±0,292	0,280
Ürea (mg/dl)	29,96±8,106	25,33±4,131	0,066
Creatinine (mg/dl)	0,87±0,186	0,89±0,163	0,496
PT	13,76±1,24	13,49±0,88	0,280
INR	1,007±0,06	1,043±0,07	0,315
qHBsAg(IU/ml)	7403 (293-32689)*	22169 (13991-80057)*	0,002
qHBsAg(xlog10 IU/ml)	3,724±0,525	4,452±0,354	0,002
HBV DNA(IU/ml)*	797246 (2015-36700000)*	131050000 (1140000-10470000000)*	0,001
HBV DNA(xlog10 IU/ml)	5,424±1,468	8,275±1,531	0,004
Fibrosis	2,36±1,31	2,67±0,516	0,034
Fibrosis < 3	13	2	>0,411
Fibrosis ≥ 3	12	4	
HAI	7,44±2,84	7,67±1,63	0,133
HAI < 9	15	4	>0,763
HAI ≥ 9	10	2	

*qHBsAg, ALT and HBV DNA levels were not compatible with normal distribution for this reason, Mann-Whitney U Test is utilized for these parameters. For other parameters Independent t test was used. * marked values refer median, min-max for related parameters

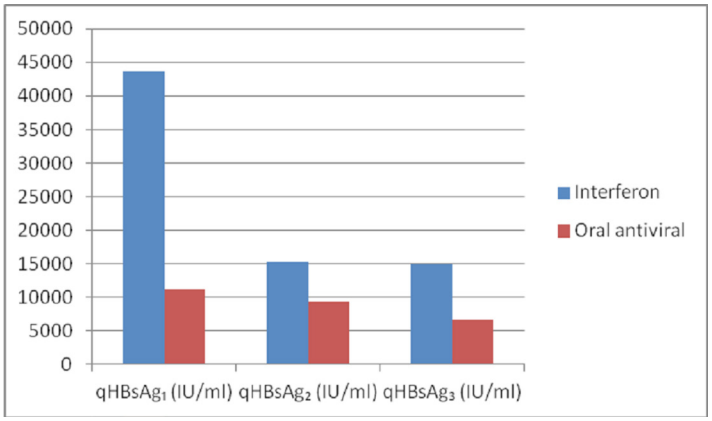
Assessment of HBsAg and HBV DNA levels during follow-up

Friedman test which was considered as a non-parametric equivalence was applied in repetitive measurements instead of variance analysis. According to the results obtained from the mentioned test, a statistically significant decrease was monitored in qHBsAg levels by time (p=0.022). While a statistically significant difference was determined between HBsAg titers at baseline and at the 3rd month of therapy (p=0.018) and between HBsAg titers at baseline and at the 6th month of therapy (p=0.007). Concordantly, while a statistically significant difference was determined in HBV DNA titers before therapy and at the 3rd month of therapy (p=0.0001) and in HBV DNA levels before therapy and at the 6th month of therapy (p=0.0001), when the 3rd and 6th months of therapies are assessed together.

When patients were assessed as patients in two separate groups who were receiving an oral antiviral drug and interferon, a statistical significant decrease was observed in the qHBsAg level as a result

obtained from the Friedman test in patients who were receiving interferon (p=0.05). On the other hand, Bonferroni corrected Wilcoxon test was used in double comparisons to determine the groups that created a difference in patients who were receiving an oral antiviral drug. According to this test, a statistical significant difference was determined between qHBsAg levels before therapy and at the 6th month of therapy (p=0.040). Distribution of are qHBsAg levels before treatment, at the 3rd and 6th months of therapy in patients who were receiving interferon and an oral antiviral drug are displayed in Table 6.

Table 6. Serum qHBsAg levels at baseline, 3th month and 6th month of CHB patients receiving peginterferon alfa-2a or oral antiviral therapy (p=0,05;p=0,040).



Scrutinizing HBsAg levels in a group of patients with chronic hepatitis B whom are under therapy for a long term

AAverage age of patients was 49.25 ± 15.227. Twenty-eight (65.2%) of the patients were females and 15 (34.8%) were males. The mean therapy duration of patients was 9.14 ± 2.66 years. Nineteen of these patients were diagnosed with chronic hepatitis B infection and received therapy for 5-10 years while 24 patients received therapy for a period of 10 years and more. Currently 27 of these patients are using a highly potent drug. Six of these patients were patients were receiving entecavir, 21 patients tenofovir, 14 patients lamivudine and two patients adefovir. When patients receiving a higher genetic barrier oral antiviral drug are compared with patients receiving a lower genetic barrier antiviral drug regarding qHBsAg levels, a statistical significant difference was determined between both two groups (p<0.05). The median value of qHBsAg in the group of patients receiving a lower genetic barrier antiviral drug was found 297.32 IU/ml (0-5063 IU/ml) while this value was 2045.68 IU/ml (0-23893 IU/ml) in the group of patients receiving a higher genetic barrier antiviral drug (Table 7). Upon closer inspection, we noticed that 19 of the patients currently receiving higher genetic barrier and potent antiviral therapy had earlier been put on lamivudine therapy which was a low potency drug.

Previously a YMDD mutation was determined in 19 patients and drug resistance was also present. Therefore, these patients were receiving highly potent antiviral with a high barrier to resistance (Tenofovir or Entecavir). Mean qHBsAg value in these patients was 2551 IU/ml (389-23893 IU/ml) while mean qHBsAg value in patients without a resistance was 1082.70 IU/ml (0-5959 IU/ml) and a statistically significant difference was determined between both two group of patients (p=0.005).

Table 7. Comparison of qHBsAg levels in CHB patients whom are under potent antiviral treatment or non-potent therapy for more than 5 years

	Potent oral antiviral use (Tenofovir or Entecavir)	N	Mean/Median*	Standart Deviation/ min-max*	P value
qHBsAg (IU/ml)	-	16	297,32*	(0-5063)*	0,006
	+	27	2045,68*	(0-23893)*	
HBsAg (xlog10 IU/ml)	-	16	2,046	1,737	0,017
	+	27	3,2391	0,992	

* qHBsAg levels were not compatible with normal distribution so for this parameter Mann-Whitney U Testi was used. * refers “mean”,”min-max” values of this parameter

Mean qHBsAg level was determined as 4422.330 ± 6053.56 IU/ml in patients who received therapy for a period of 5-9 years while mean level was determined as 1991.488 ± 2336.88 IU/ml in patients who received therapy for a period of 10 years and more whereas a statistically significant difference was monitored between the mentioned two groups (p=0.017).

There were 11 patients who previously received interferon therapy. The mean qHBsAg level in the group of patients with an interferon therapy experience was determined as 2635 IU/ml while the mean qHBsAg level was found 3213 IU/ml in the group of patients without an interferon therapy experience (p=0.712).

Mean qHBsAg levels in the group of interferon-experienced patients who previously developed an oral antiviral resistance was found 4443 ± 4191 IU/ml while mean qHBsAg levels in the group of interferon-experienced patients who previously did not develop an oral antiviral resistance was 1128 ± 1776 IU/ml (p=0.158). When both two groups were compared logarithmically in accordance to mean qHBsAg levels, qHBsAg level was found 3.49 log 10 IU/ml in the group of interferon-experienced patients who developed a lamivudin resistance. However, this value was found 1.45 log 10 IU/ml in the group of interferon-experienced patients who did not develop a resistance. A statistically significant difference was determined between both two groups regarding qHBsAg levels (p=0.010).

HBsAg seroclearance developed in 4 (9.3%) patients out of 43 who were receiving long term therapy. Two of these patients were interferon-experienced male patients and continued to lamivudine for a period more than ten years after interferon. One of the male patient out of two who developed HBsAg seroclearance received lamivudine for a period of ten years while the other patient was a female patient who received tenofovir for a period of 6 years.

Discussion

The basic function of HBsAg protein is to cover the surface of the hepatitis B virus [1]. HBV attaches to the cell membrane using the surface antigen in order to give a start and trigger the disease [1]. HBsAg is generated from HBV DNA that is integrated into the host genome using host enzymes or by translation from transcriptionally active cccDNA [1]. cccDNA is a minichromosome and by having a viral template it regenerates the HBsAg pool to sustain CHB infection [1,6]. Therefore, it is essential to appreciate the structure and function of cccDNA for a complete cure of the disease; nevertheless, evaluation and the measure of cccDNA is not easy and only liver biopsy gives opportunity for measurement of levels of hepatic cccDNA, therefore, explorations were made for a marker to reflect the quantity of cccDNA instead of only demonstrating its existence [1]. From the conducted researches, serum qHBsAg was shown to reflect the level of transcriptional activity of cccDNA [1]. In a study conducted by Volz et al, it was shown that the level of qHBsAg is higher in patients who were HBeAg positive compared to HBeAg negative patients [1,7]. In the same study, the level of cccDNA was also found to be higher in HBeAg positive patients compared to negative patients [1,7]. It can be stated as a result of these studies that the level of HBsAg is higher proportionally to the higher level of cccDNA in HBeAg positive CHB patients. In research conducted by Thomson et al., a positive correlation between qHBsAg and HBV DNA and intrahepatic cccDNA level was observed in HBeAg positive patients [1,8]. In view of these studies, it can be stated that qHBsAg level reflects cccDNA level and its transcriptional activity.

In literature, there are many trials associated with whether qHBsAg levels do or do not differentiate these phases of the disease, and by evaluating these trials, it is clearly proven that qHBsAg levels alter with the natural progression of the disease and from phase to phase [1,9,10]. While qHBsAg levels differ significantly during the four phases of infection, serum HBsAg progressively decreases from the immune tolerance to the inactive phase [1]. HBV genotypes can also influence HBsAg quantification and its kinetics. Furthermore, HBsAg decline varies by HBV genotype in PEG-IFN treated patients in response to treatment [1]. Jaroszewitcz et al demonstrated that the qHBsAg titer was significantly higher in genotypes A and D compared to other genotypes (especially in the Mediterranean region and Europe) [9, 11]. Brunetto et al. investigated whether on-treatment kinetics (peginterferon alfa-2a) of HBsAg serum levels vary by HBV genotype [1,12]. It was shown that baseline qHBsAg levels were notably higher for A versus B, C, and D genotypes ($p<0.05$) [1). On-treatment HBsAg kinetics was shown to alter according to HBV genotype [1].

There are also many studies investigating the interrelationship between qHBsAg and HBV DNA levels [1]. Most of them indicated a notably association between those markers. Ganji et al. examined the correlation between qHBsAg and HBV DNA levels in chronic HBV patients [1]. Notwithstanding the notable association found in HBeAg positive patients, no meaningful relation was shown in "HBeAg" negative patients [1,13]. In another study conducted by Antaki et al., a significant correlation between HBV DNA and qHBsAg levels was demonstrated in CHB patients who were immune tolerant and HBeAg negative [1,14]. Moreover, Alghamdi et al. also indicated a significant association between these markers in HBeAg negative patients and concluded that both of them could be considered as a predictor if they are used together [1,15]. In our study, we have also shown a significant relationship between qHBsAg and HBV DNA levels at baseline, 3rd and 6th month of antiviral therapy.

In other publications, there are also some trials evaluating the association between liver fibrosis and qHBsAg. Decreased HBsAg level was shown in patients with advanced liver fibrosis, especially in HBeAg positive chronic hepatitis B subjects most of those trials [1]. On the other hand, in only a few studies with small sample size, there was no significant relationship between liver histology and qHBsAg levels [1]. As an example, Demirören et al. conducted a study in 56 HBeAg positive patients receiving interferon alpha 2b treatment and they found no meaningful relationship between qHBsAg level and fibrosis degree [1,16]. With findings similar to this study, there was no significant relationship between qHBsAg levels and fibrosis stage although qHBsAg levels were found to be lower in patient group who had significant fibrosis ($F\geq3$).

Recent studies have demonstrated that a decrease in qHBsAg in patients receiving peginterferon may signal successful induction of immune control over HBV, and can therefore be utilized to foresee treatment response [3]. NA treatment typically induces a less rapid decline in quantitative HBsAg levels compared to patients under interferon therapy therefore, HBsAg seroconversion can be reached even in decades of NA treatment [3]. Furthermore, it was also stated that response to oral antiviral therapy will be higher if the baseline qHBsAg level is lower [1, 4]. In our study, statistically significant decrease was determined in qHBsAg levels in both patients receiving peginterferon alfa-2a and oral antiviral therapy in six-month follow-up.

Recently novel trials comparing the efficiency of peg-IFN add-on therapies with oral antiviral monotherapies has been carried out [1]. In a study by Xie et al., peg-IFN was added for a period of 48 weeks for patients who were under entacavir or tenofovir treatment and compared with patients under NA monotherapy in terms of serological response and HBeAg seroconversion [1]. For patients under Peg-IFN add-on treatment for 48 weeks, the seroconversion rate of HBeAg was shown to be higher and the decline in qHBsAg level was remarkable compared to the patients with entacavir or tenofovir monotherapy [1,17]. In another study, patients receiving peg-IFN alfa 2a and tenofovir combination therapy were compared with monotherapies in terms of HBsAg decrease [1]. Post-treatment HBsAg quantification for the combined therapy group were notably lower and HBsAg decrease was prominently higher [1,18].

When we look up the medical researches, we can see that there are only a few studies examining the association between mutated chronic HBV infection and qHBsAg levels [1). In a previous trial, quantification of HBsAg of 42 HBeAg negative patients under lamivudine treatment was observed and it was determined that if there was not a 0.7 log IU/ml decline in qHBsAg level after the six-month of therapy, then the chance of virological breakthrough can be increased to 90% [1]. Additionally, if the

patients responded to lamivudine treatment, then an important decline in qHBsAg levels in the follow-up period was indicated whereas there was no decline in patients who had lamivudine resistance [1,19]. In our study, we found statistically significant higher qHBsAg levels for the patients diagnosed with CHB if YMDD mutation and/or virological breakthrough were present in patients, who are under long term antiviral therapy compared to the non-resistant patient group. While there are only a few studies examining the relationship between resistance and qHBsAg levels, in a recently conducted study in parallel to our work it was shown that the pre-S mutations are affecting the genome replication and surface antigen expressions [20].

Conclusion

Another conclusion obtained in our study demonstrates that when the patients undergoing oral antiviral treatment for the long term (5 years and more) are divided into two groups of patients receiving either high or low genetic barrier oral antiviral therapy to resistance, qHBsAg levels for the high genetic barrier therapy patients were found to be higher than the low genetic barrier group in the manner of statistical significance (p=0,017), which was against our expectations. Upon closer inspection, we noticed that the patients currently receiving high genetic barrier and potent antiviral therapy had earlier been put on low potency treatments due and their therapy had been switched with potent antiviral drugs. For such patients with negative HBV DNA levels who are also undergoing more potent drug treatment, it was determined that the high levels of HBsAg was caused by prior drug resistance. It can thus be inferred that in patients with drug resistance, since the HBV reverse transcription cannot fix the errors it generates, a lot of mutant strains appear in HBV population in parallel to replication duration and speed, and even though viral suppression (HBV DNA negativity) is obtained by proceeding to high potency drug treatments, continuation of replications generates new mutants and make achieving of cccDNA easier, while also increasing qHBsAg levels observed on these patients. As the current oral antiviral agents used in CHB treatment target the viral polymerase, the usage of antiviral combinations or a more potent treatment will not provide any additional means to control or suppress the viremia. To say it in a better way, they will not suppress the viral replication [21]. For this reason, in patients with increased drug resistance, the use of newer antiviral agents that decrease other phases of viral replication and immunomodulator therapies that suppress cccDNA (peg-interferon etc.) along with oral antiviral therapy is thought to lower qHBsAg, thus easing HbsAg seroclerance.

This study had a few limitations. First, our study had a small sample size, which may limit the generalizability of the current results. Further research which utilizes statistical analyses to determine an appropriate sample size for sufficient statistical power is warranted to validate the current results. Another limitation for our study include a short follow-up period. We only examined the HBsAg levels at baseline, 12 weeks and at 24 weeks of naive CHB patients. As such, we suggest additional examinations utilizing qHBsAg at different times with larger sample sizes.

Quantitative HBsAg, which is the only non-invasive tool for identifying cccDNA activity, is a significant indicator that advises clinicians to the practicability of a cure of CHB infection with the elimination of viral load and cccDNA by mean of pegylated interferon and nucleoside analogs and/or new immune modulator cure methods + viral suppressive treatments [1]. Quantitative HBsAg, with its capacity to open new bases for genomic discoveries linked to Hepatitis B, will make it feasible to improve safe and more competent treatment techniques for clinicians and researchers.

Competing interests
The authors declare that they have no competing interest.

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